

CHANGES IN SERUM CALCIUM, URINE CALCIUM, SERUM PHOSPHATE AND URINE PHOSPHATE LEVELS BY CALCIUM SUPPLEMENTS IN POST- MENOPAUSAL WOMEN

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ABSTRACT

The present study was intended to find out whether the calcium supplements with vitamin D significantly affect the serum calcium, the urinary calcium, and the serum phosphate & the urine phosphate levels in post-menopausal women. Among sixty healthy postmenopausal women enrolled, thirty formed the study group (SG) with calcium supplementation for a period of a month, three months and twelve months, and the remaining thirty women formed the age-matched control group (CG) with no calcium supplementation. By using the appropriate biochemical methods the levels of serum calcium, urinary calcium, serum phosphate and urine phosphate were estimated, and the data were analyzed by using relevant statistical methods. In spite of consuming the calcium supplements over a period of time, the serum calcium levels did not vary significantly in SG, but the urinary calcium levels increased progressively (p value <0.005) in those who were administered the calcium supplements for a period of twelve months as compared to those for a month duration. There was initial decrease in serum phosphate level (after a period of 1 month of calcium intake), the serum phosphate levels gradually reached to the levels of control group, along with progressive increase in urinary phosphate levels as that in the control group (p value < 0.005). Thus, the calcium supplements, though, were of little significance on the serum calcium levels, however, have a significant effect on the levels of urinary calcium, serum phosphate and the urinary phosphate in post-menopausal women.

KEYWORDS: Calcium Supplements, Post-Menopausal Women, Serum Calcium, Serum Phosphate, Urine Calcium, Urine Phosphate.

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INTRODUCTION

Calcium plays a vital function in several important cellular physiological processes (Nordin, 1997), and the body regulates the plasma concentration of free ionized calcium (the physiologically active form of the ion) towards maintaining the plasma calcium levels within the normal range (1.0 and 1.3 mM) or (4.0 – 5.2 mg/dL). Phosphate is no less important because it is part of adenosine triphosphate (ATP) molecule. Phosphate plays a critical role in cellular energy metabolism, in activation and deactivation of enzymes. However, unlike the calcium, the plasma phosphate concentration is not very strictly regulated and its levels fluctuate throughout the day, particularly after meals.

Calcium homeostasis and phosphate homeostasis are intimately tied to each other on account of two reasons. First, calcium and phosphate are the principal components of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)(\text{OH})_2$) constituting the predominant mineral contents of bone. Second, calcium and phosphate are regulated by the same hormones, viz.

primarily PTH and 1, 25 (OH)₂ Vitamin D (Calcitriol) and to a lesser extent the hormone calcitonin. However the actions of these hormones on calcium and phosphate are typically opposed in that a particular hormone may elevate the level of one ion while lowering that of other (Eugene and Paula, 2003). Moreover, not all calcium consumed is absorbed in the GIT. Depending upon the food consumed, about 30% the calcium in foods was reported to be consumed by the humans (Committee Report, Washington DC, Institute of Medicine, 2010). Other factors also affect calcium absorption, such as (a) the amount consumed: increased calcium intake decreased the efficiency of absorption, and (b) age and life stage: in infants the calcium absorption is 60% higher, hence the significant amount of the mineral was reported to be required for the bone, (National Institutes of Health, 1994).

Because of the worries in the community about calcium insufficiency in their bone wellbeing, there has been an expanded use of calcium supplements by the postmenopausal women. Especially, the low dietary admission of calcium in elderly ladies has been accounted for to be lessening the bone mineral density (Dawson-Hudges et al., 1990; Prince et al., 1995; Reid et al., 1995), and in this manner the adequate amount of calcium should be acquired in postmenopausal women to protect the bone wellbeing (McKane et al., 1996). As of late there has been an increment in the remedy for the calcium insufficiency, and hence the augmented misuse of calcium as a medicinal means has been embroiled to numerous complications, for example, increment of gall stones, renal stones, expanded rate of myocardial infarction and different fibrotic conditions (Bolland et al., 2010). Conventional calcium supplements consumed by the old age individuals to reinforce bones might support the danger of heart attacks, recommending for revisiting the role of calcium in the treatment of osteoporosis (Cleland et al., 2010). Calcium tablets have been normally endorsed to support skeletal wellbeing, yet late studies (Reid et al., 2006; Bolland et al., 2008) proposed that they might raise the rates of myocardial infarctions and cardiovascular events even in healthy old age women. Cleland et al., (2010) called attention to that paying little mind to influencing effects on heart attack rates; calcium supplements were likely not exceptionally proficient in decreasing fractures. In community-inhabiting postmenopausal women without any manifestations of bone ailment, for example, osteoporosis, every day supplementation with 1,000 mg or less of calcium and 400 IU or less of vitamin D did not avoid bone fractures (Moyer, 2013). The balance of calcium has additionally been accounted for to be altogether affected by the adjustments in the amount of calcium discharged in the urine (Heaney, 1996) and studies (Yoshida et al., 2012; Heinrich et al., 2008) reported the relationship of urinary calcium with consumption of calcium and therefore the urinary calcium can be used as an index of calcium ingestion. Although the literature abounds with the data on intestinal calcium assimilation, calcium metabolism, and calcium adjustments, the data needs a simple, observational relative study on calcium supplementation influencing the calcium phosphate interrelationship in postmenopausal ladies. Therefore, we have performed an age-matched comparative investigation of the calcium supplementation with reference to the serum calcium, the urinary calcium, the serum phosphate and the urinary phosphate levels among the supplemented and non-supplemented postmenopausal women. This study is basic, observational relative study, not tedious, with no follow-up, just with estimation of serum and urinary calcium levels, and serum and urinary phosphate levels, while the components like vitamin D and PTH hormonal measures and serum estrogen levels are being the restraints of the present study.

MATERIALS AND METHODS

Subjects

The subjects enlisted in the study were postmenopausal ladies going to the out-patient ward, Orthopedics, Osmania General Hospital, Afzal Gunj Hyderabad, Andhra Pradesh, India. The participants for this study - browsed the OP

register - were the individuals who had already consumed calcium supplements with vitamin D. They availed the supplements from the hospital pharmacy (dicalcium phosphate with vitamin D). The subjects for this study were 60 (30 study group and 30 control group) postmenopausal women of the age between 48-60 years. Women with a background marked by disorders, for example, diabetes, hypertension, malignancies, and renal ailments were excluded ineligible similar to those with some other restorative conditions that influence calcium metabolism. The women included for this study were those who were not on hormone substitution treatment, not with bone fractures, and not had been the regular users of anabolic steroids, glucocorticoids, anticonvulsants or any other medications known to influence calcium metabolism for the previous twelve months. The healthy postmenopausal women were enlisted in view of the background and other routine clinical examinations and allocated to calcium study group (SG) on calcium supplementation and the control bunch (CG) not on calcium supplementation. The SG comprised of thirty postmenopausal women, the individuals who were on calcium supplements with vitamin D for a month, up to three months, and up to twelve months (measurements: each uncoated tablet-dicalcium phosphate IP-0.5 gm; vitamin D3 IP – 500 universal units; 2 tablets OD). While the age-controlled control group of thirty subjects were not given any placebo and encouraged to proceed with their routine diet and way of life.

Ethics Approval

Ethical approval was endorsed from the ethical committee of Osmania Medical College, Hyderabad, before beginning. Informed written consent was obtained from every member before partaking in the study.

Data Collection

The subjects were allocated utilizing our own organized approved questionnaire at the beginning of the study. Written consent was taken from every one of the subjects subsequent to clarifying them about the reason and utilization of study.

Sample Collection

The blood and urine samples were collected from both the SG and the CG for estimation of serum calcium, urine calcium, serum phosphate and urinary phosphate. The subjects were studied while taking their typical eating routine without particular dietary suggestions, yet were requested to continue fasting from 9 pm the day preceding sample collection. The blood samples and 24 hour urinary samples were collected the next morning at 8 am in sterile, clean dry containers, and stored at - 20°C

Estimation of Serum Calcium Levels

Serum levels of calcium were measured by using calcium-kit method (Smith Jr and Baucer, 1979; (Budesinky, 1969; Cadwell, 1970) - calorimetric method using Arsenazo III.

Estimation of Serum Phosphorus Levels

Serum levels of phosphorus were measured by using the phosphorous kit-method (Daly, 1972; Gamst and Try, 1980; Amador and Urban, 1977) - UV-end point method using ammonium molybdate

Estimation of Urinary Calcium Levels

The 24 hourly collected urine samples were assigned for estimation of urinary calcium by using calorimeter based calcium-kit method by using Arsenazo III.

Estimation of Urinary Phosphorous Levels

The 24 hourly collected urine samples were assigned for estimation of urinary phosphorous levels by using the phosphorous kit-method (Daly, 1972; Gamst and Try, 1980; Amador and Urban, 1977) - UV-end point method using ammonium molybdate.

Statistical Methods

The statistical analyses were performed by contrasting the information in different subgroups of SG and CG and the measures of serum calcium, urinary calcium, and serum phosphate and urinary phosphate were performed by utilizing analysis of variance for multiple measurements. The biostatistical assessment was completed utilizing the statistical routine, SPSS form 18.0 (PASW statistics) for windows, (SPSS, Inc.; Chicago, IL).

RESULTS AND DISCUSSIONS

The relative data for serum calcium, urine calcium, serum phosphate and urinary phosphate among the study group and the control groups are presented in **Table 1**. The mean and the standard deviation values for the serum calcium in both the control group and the study group exhibited little variation. There was also little variation in values of serum calcium within the study group itself in spite of supplementation for the duration of more than 3 months.

The outcomes for analysis of variance (**Table 2**) indicated little significance for serum calcium in both SG and CG. In the study group, despite the fact that there was a consistent supply of the same amount of calcium, there was no significant change of serum calcium. Hence, it is suggested that in SG and CG of the post-menopausal women in the same age and with the same incorporation and avoidance criteria, the impacts of hormones on calcium metabolism are apparent to be the same. Such a perspective was steady with a reported study (Dawson-Hughes et al., 2009) that, no undeniable side effects were realized in the short term with insufficient ingestion of dietary calcium from food and supplements, as the circulating blood levels of calcium were firmly controlled and did not change with little adjustments in dietary calcium intakes. The comparative assumed hormonal impacts on calcium metabolism from our results additionally support the certainty (Goldman, 2007) that as for calcium, the homeostatic framework was adequately adaptable to keep up blood ionized calcium within the normal range without being hindered by wide variances in dietary calcium ingestion and changing rates of bone mineralization.

The urinary calcium levels dynamically enhanced in SG as contrasted with those in the CG. Indeed, even in SG, the urinary calcium levels were high in those subjects who were administered with calcium supplements for twelve months. The analysis of variance results (**Table 2**) also likewise demonstrated a statistical significance ($p < 0.05$) for urinary calcium in SG, whereas there were no measurably noteworthy variations in CG. Nevertheless, just two subjects among the thirty of the SG displayed lower levels of urinary calcium. This finding of urinary calcium just in two subjects may not be of much essentialness to the general study. This might presumably be because of the impact of PTH hormone and Vit D, which were the major restraints of our study. Additionally, there might be the likelihood of minor mistakes amid the sample collection, transport and storage, and estimation of urinary calcium levels amid the study.

There was an enduring increment in urinary calcium levels comparing to the duration of ingestion of calcium supplements (i.e. more urinary calcium levels in subjects taking calcium supplements for over three months contrasted with those taking for one month). These expanded levels in urinary discharge of calcium may indirectly reveal the elevation of the renal limit for discharge. As the urine sample was collected following 24 hours of admission of last dosage of calcium supplements, the amount of calcium absorption and later spilling in urine is uncertain, offering approach to different intricacies, for example, build up in the delicate tissues, henceforth empowering the extension for controlling the unpredictable utilization of calcium supplements in post-menopausal women.

The Mean and SD of serum phosphate levels (**Table 2**) in CG were compared with those of serum phosphate levels in the SG (1 month, 1-3 months, and >3 months). It exhibited the value of 3.1 in 1 month to 3.7 in 1-3 months, then to 3.9 in >3 months slowly reaching the mean of 4.4 in CG. Also SD from 0.6 to 0.8 to 0.5 in study group trying to reach 0.6 in the control group. This showed a gradual restoration of serum phosphate levels to those of the levels in CG over a period of time. Therefore, it indicated a decrease in the serum phosphate levels in the beginning, but exhibited a gradual restoration to the levels of control over a period of time in SG. The amount of phosphate excreted in urine was corresponding to that of serum phosphate (**Table 1**). As the subjects in SG and CG were post-menopausal women with the same consideration and avoidance criteria and age-matching, the impacts of hormones on calcium homeostasis was apparent to be the same. In lieu of the restraints of our study, the estimation of hormones during this period was not attempted to.

As stated earlier, in case of CG there was no statistically significant variation of serum calcium and urinary calcium. In SG even though there was a constant supply of the same amount of calcium, there was a progressive increase in the amount of calcium excretion with no significant alteration of serum calcium, but there was a significant decrease of serum phosphate in the beginning and a gradual restoration to the levels of the control group over a period of time. The initial decrease of serum phosphate may be reflecting the transient disturbance in a calcium-phosphate interrelationship due to calcium supplements. Therefore the gradual returning of serum phosphate to the levels that of control group was presumed to be an indication of the restorative time frame of calcium and phosphate levels. After a period of over three months and above, the levels of serum phosphate significantly reached to the levels of the control group, that is the calcium and phosphate interrelation was restored significantly, but still it was incomplete; the values of the Mean and SD of serum phosphate in SG where the levels were almost trying to reach the levels in control group gradually over a period of time, i. e. little near to levels in the control group after 1 month, then little more from 1-3 months, almost nearing after 3 months and above, but still not reaching the same levels as in the control group. Hence the calcium-phosphate interrelation was restored, but still incomplete though significant.

Despite a normal physiological reciprocal-relationship between the serum calcium and the serum phosphate levels, the serum phosphate levels were decreasing at the beginning with no corresponding increase in the serum calcium levels (Table 1). The increased levels in urinary excretion of calcium indirectly reflect the transient increase of serum calcium with calcium supplements. This is because of the fact that the more dose of calcium (through calcium supplements taken over a period of time) might have caused more calcium getting absorbed from the gut and getting expelled in urine. This indirectly reflects the transient increase of serum calcium with supplements. Table 1 also showed a progressive increase in urinary phosphate along with serum phosphate levels reaching to the levels of the control group during the period of study (i.e. over a period of time of 1 month, 1 - 3months, and >3 months)._If calcium supplementation was more

than the renal threshold levels of excretion, there could be a danger of calcium deposition in various soft tissues, causing various disorders like increased incidence of calcium stone formation in the gall bladder or kidney and increase incidence of myocardial infarction.

CONCLUSIONS

There was no critical change in serum calcium levels in the SG even after utilization of calcium supplements for a period of twelve months, while the urinary calcium levels dynamically elevated just in SG and not in CG. This implied the calcium supplementation in the long run had little impact on serum calcium levels yet essentially influencing the urinary calcium levels. The significant dropping off in the serum phosphate levels at the beginning of the study also could not reciprocally increase the serum calcium levels. The initial decrease of serum phosphate and gradual restoration to the levels of CG might be reflecting the transient disturbance in the calcium-phosphate interrelationship due to the calcium supplements in post-menopausal women.

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APPENDIXES

Table 1: Comparison between the Control and Subject Groups Depending on Period of Intake of Calcium

	Control Group (Not on Calcium Supplementation)		Study Group (Calcium Supplementation)					
			Period of Intake of Calcium					
			1 Month		3 Months		12 Months	
	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation	Mean	Standard Deviation
Age	51	4	52	4	50	1	51	1
Serum Calcium (mg/dl)	8.6	0.3	8.5	0.2	8.5	0.3	8.9	0.5
24 hrs Urinary calcium (mg/dl)	131	14	142	39	192	15	237	13
Serum phosphorous (mg/dl)	4.3	0.6	3.0	0.5	3.6	0.8	3.8	0.5
24 hrs Urinary phosphorous (gm/dl)	0.98	0.08	0.55	0.33	0.55	0.25	0.81	0.27

Table 2: Results Analyses of Variance with Mean Values of Serum Calcium, Urinary Calcium and Serum Phosphate and Urinary Phosphate

		Sum of Squares	Df	Mean Square	F*	Sig.
Serum Calcium (mg/dl)	Between Groups	0.543	3	0.181	1.584	0.203
	Within Groups	6.381	56	0.114		
	Total	6.923	58			
24 hrs urinary calcium (mg/dl)	Between Groups	83710.8	3	27903.6	55.85	0.000
	Within Groups	27974.5	56	499.54		
Serum phos(mg/dl)	Total	111685.4	59			
	Between groups	17.65	3	5.885	14.104	0.000
	Within groups	23.36	56	417		
24 hrs urinary phos gm/day	Total	41.02	56			
	Between groups	2.195	33	0.732	15.090	0.000
	Within groups	2.714	55	0.048		
	Total	4.911	58			

*Asymptotically F distributed